Supplementary Material

for

Adult neural stem cells in distinct microdomains generate previously unknown interneuron types

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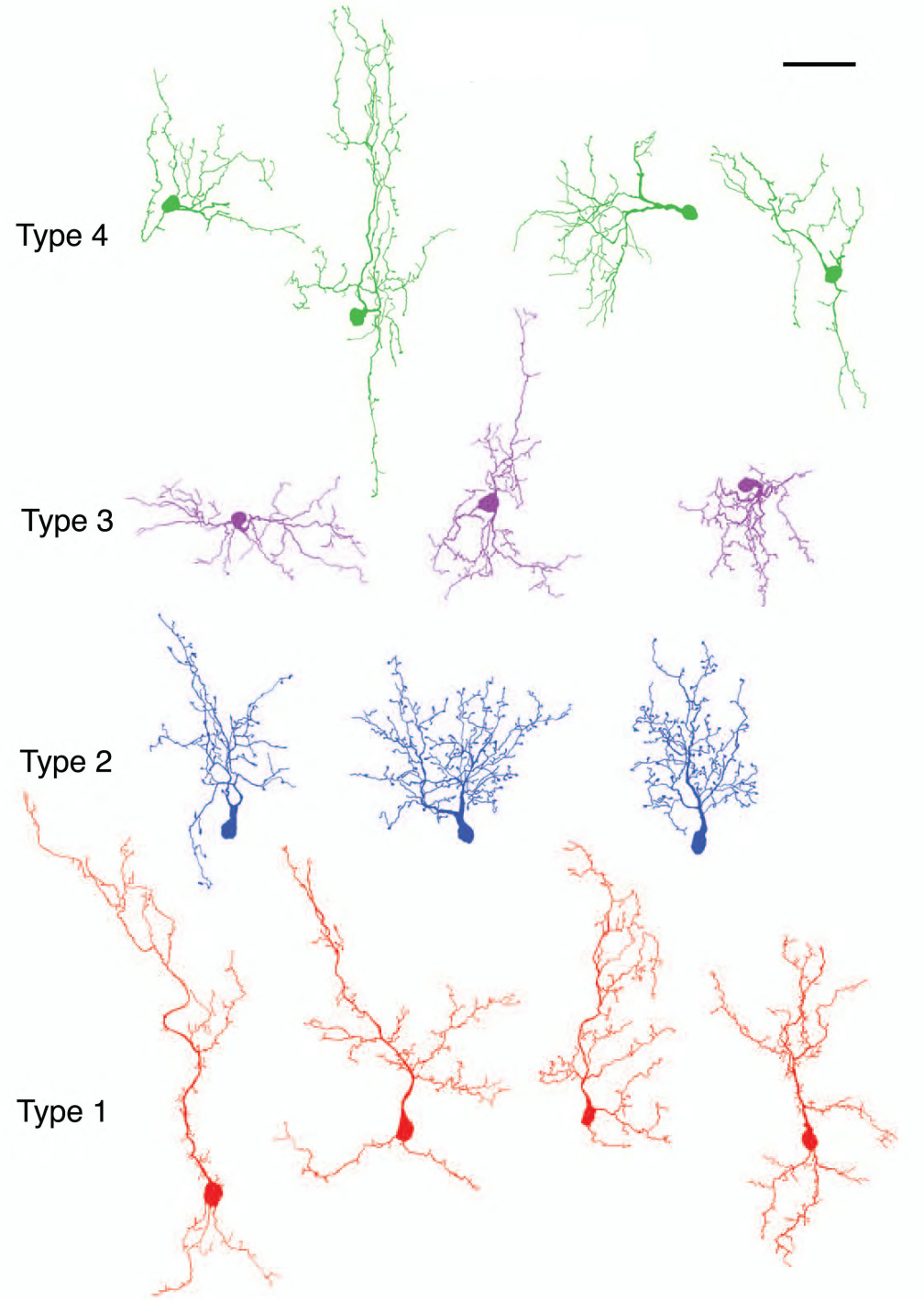
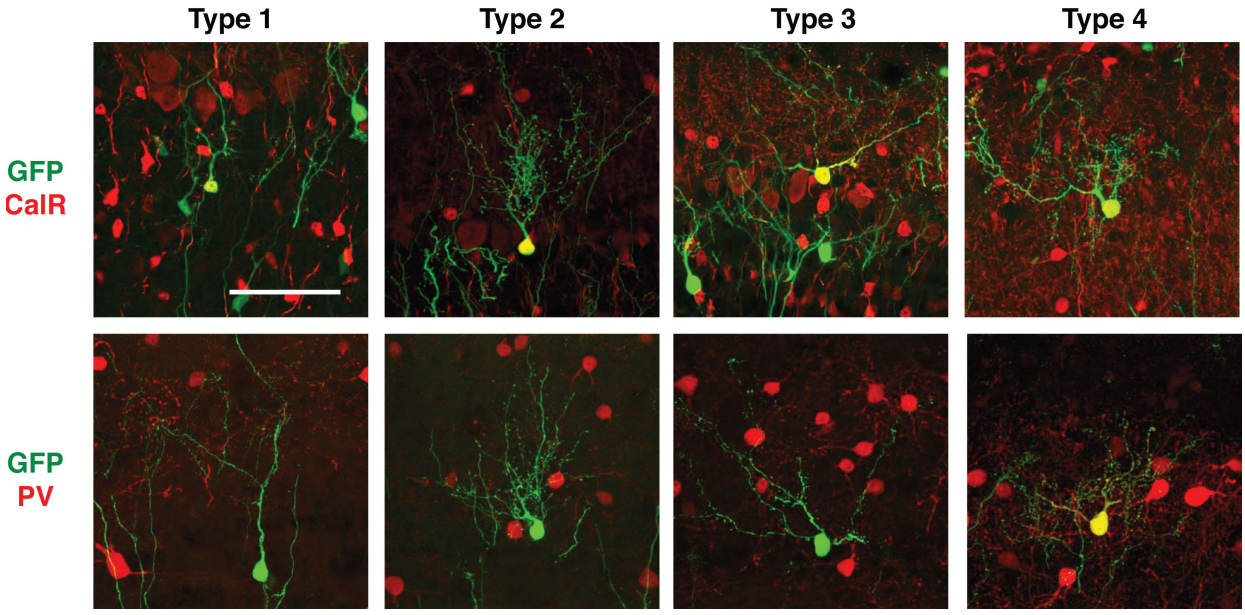
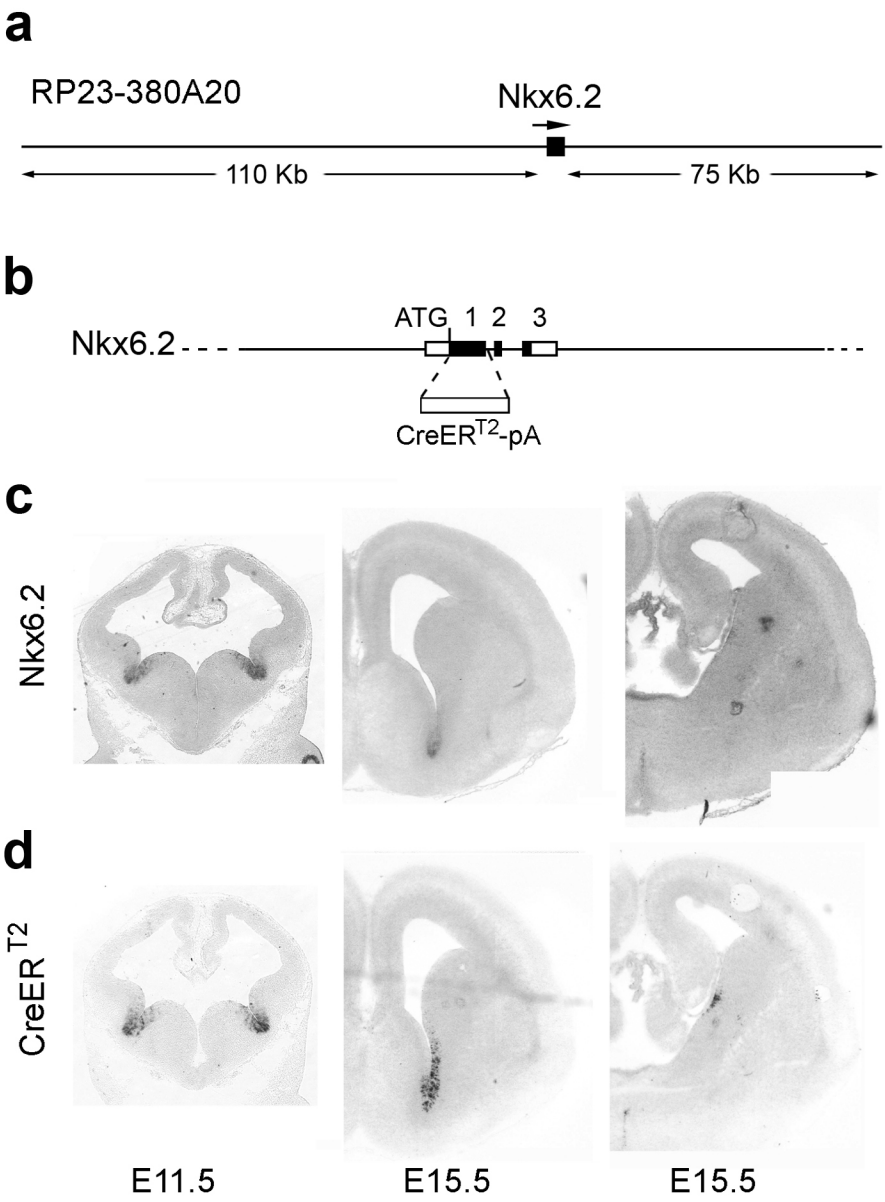


Supplementary Figure 1. Type 1-4 cells have unique morphologies. All cells are shown to scale and were traced using a camera lucida, digitized, and colored. **Type 1** cells (red) resemble known granule cell types, but have cell bodies located in the superficial GRL and dendrites restricted to the deep layers of the EPL and below. **Type 2** cells (blue) have cell bodies in the MCL and a spatially restricted, superficially directed, and highly branched dendritic arbor. **Type 3** cells (magenta) have cell bodies in the MCL and relatively thin but highly branching processes concentrated in the IPL and MCL. **Type 4** cells (green) are located in the EPL and have branched dendritic arbors with processes that tend to align vertically. Scale bar is 50 µm.



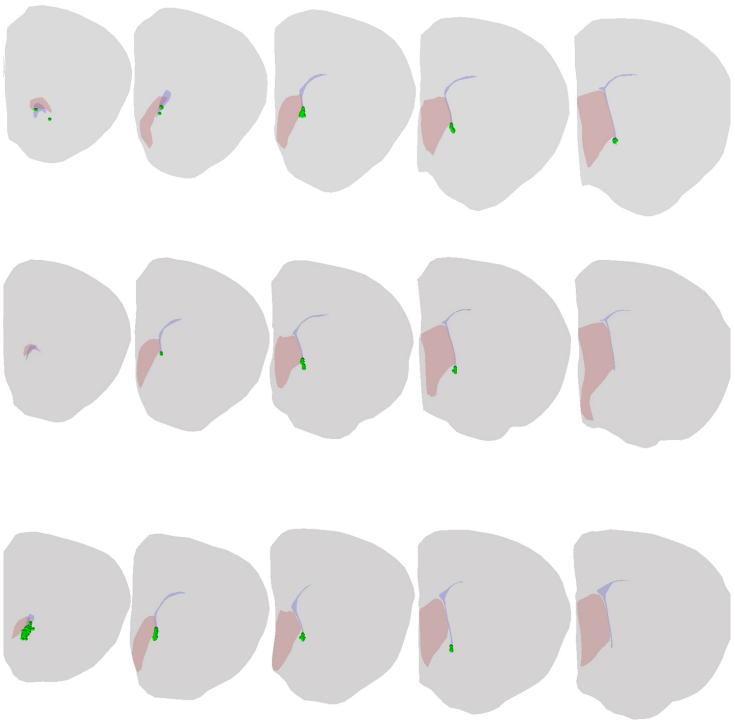
Supplementary Figure 2. Type 1-4 cells express markers of interneurons. A subpopulation of Type 1-4 cells strongly expresses the calcium binding protein and interneuron marker calretinin (CalR). Type 1-4 cells were negative for parvalbumin (PV), though rare cells in the EPL are clearly immunopositive for PV (pictured). These cells were larger than Type 4 cells and likely respond instead to Van Gehuchten cells. Scale bar for all photomigrographs is 50 μm.



Supplementary Figure 3. Generation and characterization of *Nkx6.2::CreER^{T2}* mice

a,b) Strategy for the generation of mice expressing CreER^{T2} under control of Nkx6.2. Structure of the unmodified genomic BAC used for generation of the transgene (a) and modification of the genomic BAC containing the *Nkx6.2* gene by insertion of iCreER^{T2}-polyA within the first coding exon (b). **c)** RNA *in situ* hybridization showing expression of *Nkx6.2* at E11.5 and E15.5. **d)** RNA *in situ* hybridization showing expression of the *CreER^{T2}* transgene at E11.5 and E15.5. The endogenous *Nkx6.2* gene and the transgene are both expressed in the interganglionic sulcus at E11.5. At E15.5, the transcripts can still be detected in the sulcus but strong expression can also be observed in the V-SVZ.

a

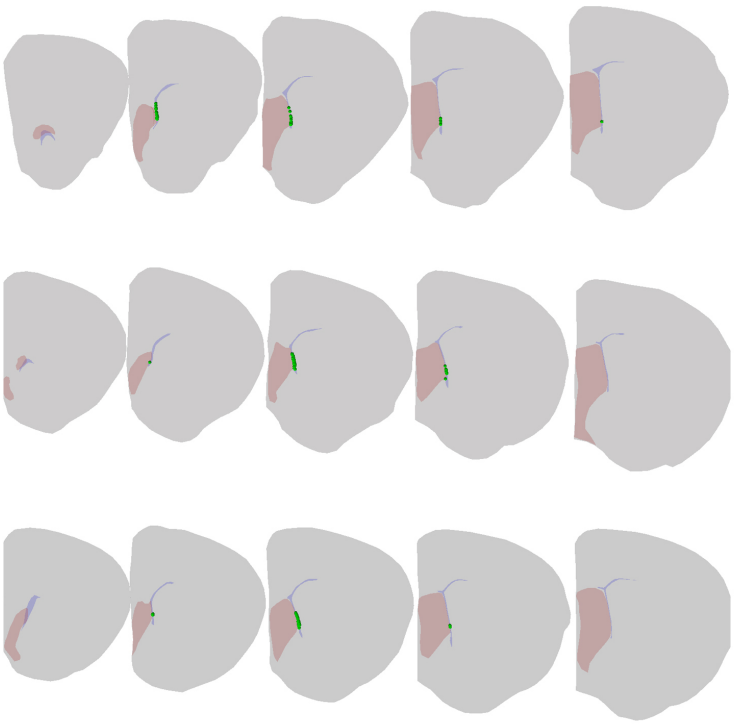


P361
Zic^{+}PGCs = 30.8%
PGC/GC = 0.1
% Type 1 = 0.4%
% Type 3 = 0.4%

P373
Zic^{+}PGCs = 33.3%
PGC/GC = 0.2
% Type 1 = 0%
% Type 3 = 0%

P411
Zic^{+}PGCs = 29.7%
PGC/GC = 0.6
% Type 1 = 0.5%
% Type 3 = 0.4%

b

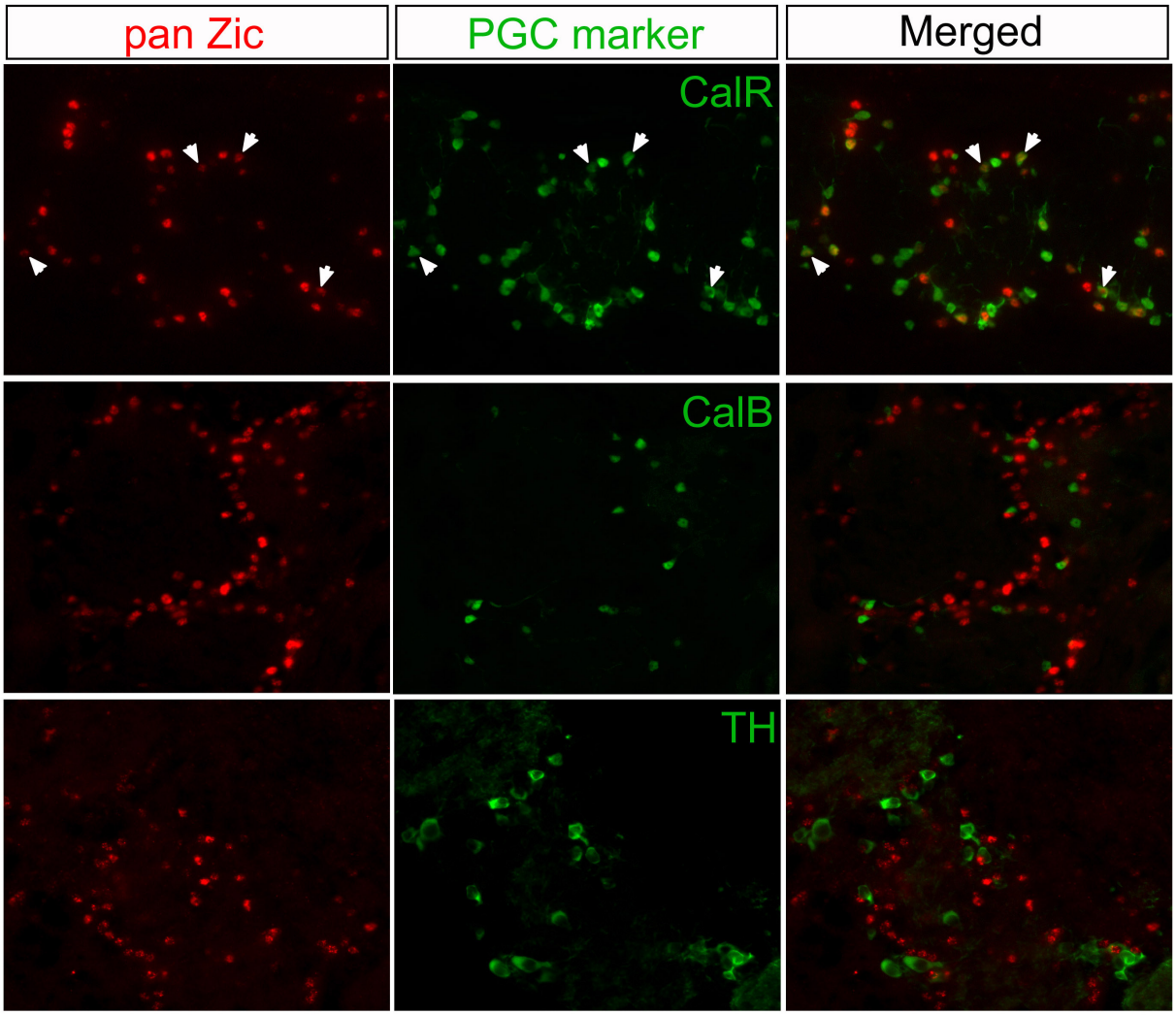


P365
Zic^{+}PGCs = 91.7%
PGC/GC = 14.7
% Type 1 = 0.5%
% Type 3 = 1.0%

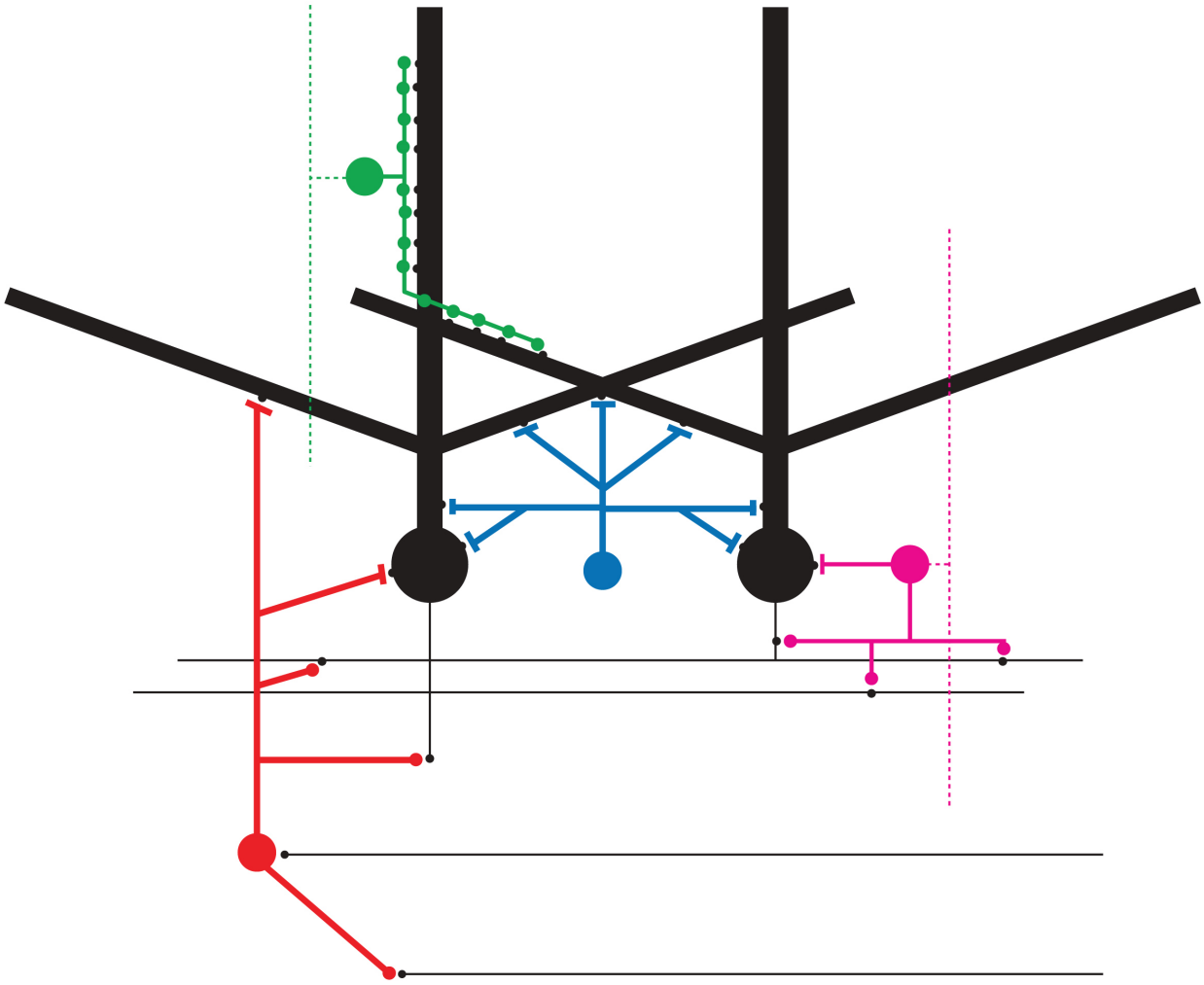
P392L
Zic^{+}PGCs = 97%
PGC/GC = 2.5
% Type 1 = 0%
% Type 3 = 2.4%

P396L
Zic^{+}PGCs = 93.5%
PGC/GC = 3.1
% Type 1 = 3.4%
% Type 3 = 1.7%

Supplementary Figure 4. Zic immunopositive OB interneurons are generated in a medial and anterior domain. Neurolucida traces of coronal sections from Z/EG mice brains injected at P0 with Ad:Cre to target radial glia on the medial wall of the anterior ventral V-SVZ. The surface of the brain is colored in gray, the lateral ventricle is shown in light purple, and the domain containing Zic immunopositive cells is shown in light red. Radial glial-derived (GFP+) V-SVZ cells are indicated in bright green. Injections were then classified into two groups (a and b) based on the ratio of periglomerular to granule cells in the OB (PGC/GC). As previously described, high ratios (>2) correlated with the presence of more rostrally located GFP+ cells in the V-SVZ. **a)** The more posterior labeling group had low PGC/GC ratios and intermediate percentages of Zic immunoreactivity among PGCs. Labeling in the V-SVZ was concentrated near the ventral tip of the lateral ventricle. **b)** The more anterior labeling group was characterized by high PGC/GC ratios and a high percentage (>90%) of PGCs that expressed Zic. Furthermore, the vast majority of Type 1 and Type 3 cells derived from this domain were Zic+.



Supplementary Figure 5. Zic is expressed in a subset of CalR+ PGCs. Double immunostaining for Zic and markers of PGC subtypes demonstrates co-labeling among Zic and CalR, but very little overlap with CalB or TH. This result is consistent with the previously identified medial anterior domain of CalR+ PGC generation. The presence of a Zic-/CalR+ population is consistent with the observed origin of CalR+ PGCs from other regions such as the cortical V-SVZ, whereas the presence of Zic+/CalR- cells suggests the presence of additional interneuron subtypes among the Zic+ population.



Supplementary Figure 6. The location and morphology of Type 1-4 cells suggests unique key roles in OB function. Here we speculate as to what roles Type 1-4 cells might play in the OB circuitry, bearing in mind that these hypotheses must be tested in future experiments. Type 1 cells (red) may receive axonal (possibly dendritic) input within the superficial granule cell layer and internal plexiform layer and inhibit the cell bodies and proximal dendrites of mitral (black) and tufted cells above them, thereby mediating columnar inhibition. The highly branched, spatially restricted arbors of Type 2 cells (blue) are positioned to inhibit the cell bodies and proximal dendrites of neighboring mitral and deep tufted cells and could mediate localized lateral inhibition. The varicosities and spines of Type 3 (magenta) and 4 cells (green) may be sites of unidirectional (pre or post-synaptic only) or reciprocal synapses. If they are post-synaptic, Type 3 and 4 cells may detect the output of mitral and tufted cells or local processing in their dendrites and relay this activity to other cells in the column via their radially projecting axons. If they have reciprocal synapses or pre-synaptic structures, Type 3 and 4 cells may inhibit the output of mitral and tufted cells or inhibit their dendrites, respectively.